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COMPARING THREE MTHODS OF ASSAYING SOIL FUSARIUM AND TRICHODERMA POPULATIONS FOR INTEGRATED PEST MANAGEMENT IN FOREST NURSERIES

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ABSTRACT

Three methods for assaying soil populations of potentially pathogenic *Fusarium* spp. and potentially antagonistic *Trichoderma* spp. were compared at the USDA Forest Service Lucky Peak Nursery near Boise, Idaho. Standard soil dilutions and estimates of colonization of residual roots within soil and roots from healthy-appearing seedlings were compared at the end of the first growing season of 1-0 ponderosa pine. Tested areas had undergone four different pre-plant treatments (methyl bromide/chloropicrin fumigation, fallowing with periodic cultivation, and incorporation of two different *Brassica* green manure crops). The three assay methods were similar in their ability to estimate treatment effects on soil populations of both *Fusarium* and *Trichoderma*. However, all three methods did not give adequate predictions of disease potential because they estimated overall *Fusarium* populations instead of elaborating pathogenic isolates. Improved assay methods for implementing integrated pest management should include molecular markers for pathogenic strains of *Fusarium* to supplement soil and seedling root colonization assays.

INTRODUCTION

Most disease control in forest nurseries involves preventing disease rather than treating disease once it becomes apparent. To prevent disease, cultural manipulations are made on the pathogen itself and/or on the conditions conducive to disease. Because of this emphasis on prevention, fairly high levels of pesticides have traditionally been used. In particular, pre-plant soil fumigants have become common in bare root nurseries due to improved stock quantity and quality that normally results from such treatments (Boone 1988; Boyd 1971; James 1989). Chemical soil fumigants are non-specific biocides that kill all organisms within treated fields (Ebben et al. 1983; Fuller et al. 1980; Johnson and Zak 1977). Although pathogen levels are usually eliminated or reduced by fumigation, beneficial microbes are also adversely affected (Ibarbia 1995; James 1989; Munnecke et al. 1978). As a result, biological balances of microorganisms are disrupted and growers often have to repeat fumigations for each subsequent seedling crop (James 1989; Miller and Norris 1970). If fumigations are not conducted, high pathogen populations usually develop and often maintain themselves for several years until biological balances are naturally restored (Danielson and Davey 1969; Marois et al. 1983; Vaartaja 1967).

Some nurseries have continually produced high-quality bare root seedlings without pre-plant chemical soil fumigation (James 1989). In these situations, soil microbial populations may become suppressive to pathogens (Amir and Alabouvette 1993; James 1989). Under

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suppressive conditions, pathogens may be present, even at relatively high levels, but do not elicit disease (Alabouvette et al. 1979; Amir and Alabouvette 1993). Many factors may affect soil suppressiveness, but one of the most important is quantity and makeup of microbes that are antagonistic toward pathogens (Abdul Wahid et al. 1998; Alabouvette et al. 1979, 1984). Disease suppressiveness is eliminated with soil fumigation (James 1989).

If efforts to reduce or eliminate use of chemical pesticides, including pre-plant soil fumigation, are successful, growers will have to adopt a system of integrated pest management that will combine several different approaches to keep pest populations low (Linderman et al. 1994). However, for effective integrated pest management, good systems of pest population monitoring are essential (Bruehl 1989; Delahaut and Koval 1994). Monitoring is necessary to ascertain when population thresholds are exceeded so that satisfactory control measures may be instituted (Bruehl 1989; Heinz and Parrella 1991).

Since one of the major problems confronting production of high-quality forest tree seedlings in bare root nurseries is soilborne pathogens inciting root disease (Bloomberg 1976; Smith and Bega 1966), monitoring soil populations of potentially-pathogenic organisms is a necessary prelude to effective integrated pest management (Bruehl 1989). The standard procedure for estimating soil populations of pathogenic fungi is soil dilution analysis from samples onto a selective agar medium for the group of targeted organisms (James and Beall 1999, 2000; James et al. 1996). This procedure requires laboratory analysis of soil fungi enumerated onto agar plates. Also, it assays all propagules within the target group rather than focusing on those isolates capable of eliciting disease (James et al. 2000). Other laboratory approaches include assaying for potential pathogens residing within residual roots that may be left from either previous seedling crops or produced from root pruning during crop production (James and Perez 1999). This procedure narrows the assay to those microorganisms capable of colonizing root tissues (James and Perez 1999), since these are of more concern from a disease potential standpoint (Bloomberg 1976; Bloomberg and Lock 1972; Booth 1966; James et al. 1991). Another approach is to assay for root colonization on seedlings that do not display disease symptoms (James 2002a, 2002b; James and Gilligan 1988a, 1988b). Since many potentially pathogenic fungi routinely colonize root cortical cells prior to initiating disease (Booth 1966; James et al. 1991, 1994), assaying of attached seedling roots limits the population sampling to those organisms most likely capable of causing disease.

These three approaches to pathogen population monitoring have been utilized in the past at different nurseries. However, their efficacy in determining disease control treatment effects within a single area has not been compared. Therefore, the three methods were applied to same field and seedlings and quantitatively compared for the elucidation of potential pathogen populations.

MATERIALS AND METHODS

Three methods of assaying for potentially-pathogenic fungi in a bare root nursery were compared at the USDA Forest Service Lucky Peak Nursery, near Boise, Idaho. This nursery has been the site of several previous evaluations of the effectiveness of various treatments to reduce impacts caused by soilborne pathogens in general and *Fusarium* spp. in particular (James and Beall 1999, 2000). *Fusarium* spp. are important causes of both damping-off and root diseases within conifer seedling crops grown at the nursery (James 2001, 2002b).

All assay methods were conducted from samples collected from the same fields that had undergone four different pre-plant soil treatments. These treatments included: (1) standard soil fumigation with methyl bromide/chloropicrin (67% methyl bromide 33% chloropicrin – applied at 350 lbs./acre), (2) bare fallowing with periodic cultivation for one

growing season prior to sowing, (3) *Brassica* (dwarf essex) green manure crop grown during the fall and winter prior to sowing (incorporated at least 14 days before sowing), and (4) *Brassica* (mustard) green manure crop grown during the fall and winter prior to sowing (incorporated at least 14 days before sowing). All sampling was conducted during the fall at the end of the first seedling growing season (figure 1).

Soil Populations

Six soil samples were collected within plots from each treatment. Samples were collected about in the middle of each plot between seedling rows. At each sample point, a soil core was taken to a depth of about 8 in. (20 cm). Soil was placed in plastic bags, kept refrigerated, and transported to the laboratory for analysis.

Standard soil dilutions (James and Beall 1999, 2000, James et al. 1996) were conducted to estimate populations of *Fusarium* and *Trichoderma*. Soil from each sample was initially sieved (2 mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5g subsample was oven-dried at about 100^{o}C for at least 24 hrs. until sample weight stabilized. Oven-dry weight was then calculated to provide a standard for sample comparison. For assays of *Fusarium* and *Trichoderma* populations, 0.05g of field-moist soil was combined with 10 ml of 0.3% water agar and thoroughly mixed. One ml of the solution was then placed on each of three plates of a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975) and spread uniformly. Plates were incubated 5-7 days at about 24^{o}C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium; populations were expressed as colony-forming units (cfu) per g of oven-dried soil. Selected *Fusarium* isolates were transferred to potato dextrose (PDA) and carnation leaf (CLA) agar (Fisher et al. 1982) for identification using the taxonomy of Nelson et al. (1983). Average populations for each of the four pre-plant treatments were calculated.

In addition, average ratios of *Trichoderma* to *Fusarium* populations (T/F ratio) were calculated to give an rough estimate of potential disease suppressiveness (James 2002a, 2002b). In general, the higher the T/F ratio, the less *Fusarium*-incited disease can be expected.

Average soil populations of *Fusarium* and *Trichoderma* were statistically compared among the four pre-plant treatments using an analysis of variance. Significantly different ($P \leq 0.05$) means were located using Tukey's HSD.

Residual Root Colonization

Assaying residual (detached) roots within soil has previously been done to enumerate populations of *Fusarium* within soil that might be capable of causing disease (James and Perez 1999). Detached roots within soil were extracted from collected soil samples during sieving (see above). The roots were washed thoroughly to remove adhering soil particles, surface sterilized for 1 min in a 10% bleach solution (0.525% aqueous sodium hypochlorite) and rinsed in sterile, distilled water. Roots were cut into pieces approximately 3-5 mm in length and placed on Komada's medium and incubated as described above. One hundred twenty root pieces were assayed from each of the four treatment areas. Selected *Fusarium* isolates were reisolated and identified as described above. Percentage of root pieces colonized by *Fusarium, Trichoderma*, other (unidentified) fungi or no fungi were determined. Percent root colonization by the different groups of fungi among the four pre-plant soil treatments were compared statistically with an analysis of variance. Significantly different ($P \leq 0.05$) means were located using Tukey's HSD. All percentages underwent arc-sin conversion prior to analysis.

Seedling Root Colonization

Seedling root colonization by potentially pathogenic fungi has been a useful measure of pathogen populations and an estimate of disease potential (James 2002a, 2002b; James and Gilligan 1988a, 1988b). Fifteen nondiseased seedlings were randomly collected at the end of the first growing season from each pre-plant treatment area. Seedlings were carefully extracted to make sure most of their roots were collected and transported to the laboratory for analysis. Root systems were washed thoroughly under running tap water to remove adhering soil particles. Ten root pieces located at the terminal ends of lateral roots were randomly selected and excised from roots; pieces were approximately 3-5 mm in length. Root pieces were surface sterilized, rinsed and incubated on Komada's medium as described above. Selected *Fusarium* isolates were reisolated and identified as described above. Percentage of root pieces colonized *by Fusarium, Trichoderma, Cylindrocarpon* and other (unidentified) fungi were determined. Percent root colonization by the different groups of fungi among the four pre-plant soil treatments were compared statistically with an analysis of variance. Significantly different ($P \leq 0.05$) means were located using Tukey's HSD. All percentages underwent arc-sin conversion prior to analysis.

Figure 1. 1-0 bare root ponderosa pine seedlings at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho. White stakes delineated plots for sampling soil, residual roots and healthy seedling roots. *Fusarium*-caused mortality is indicated by arrows.

RESULTS

Soil Populations

Soil populations of *Fusarium* and *Trichoderma* spp. among the four pre-plant treatments are summarized in table 1. Treatment with methyl bromide/chloropicrin resulted in significantly ($P=0.05$) lower *Fusarium* and significantly higher *Trichoderma* populations by the end of the first seedling growing season. Likewise, the other three treatments resulted in high ($>$ 1000) or very high ($>$ 3000) populations of *Fusarium* and corresponding low populations of *Trichoderma*. This resulted in very low T/F ratios for all treatments except soil fumigation (table 1).

By far the major *Fusarium* species isolated from soil was *F. oxysporum* Schlecht. (table 2). This species comprised nearly 94% of all the *Fusarium* isolates obtained from soil. Four different morphological strains (morphotypes) of *F. oxysporum* were isolated, three of which (types 1, 2 and 4) were present in fairly high numbers. The fourth morphotype (3) was isolated infrequently; this type consisted of an appressed, pionnotal type that had abundant macroconidia, but fewer microconidia. Other *Fusarium* species isolated from soil included *F. equiseti* (Corda) Sacc. *F. chlamydosporum* Wollenw. & Reinking and *F. acuminatum* Ell. & Ev. All these species were present at relatively low levels except for *F. equiseti*, which was isolated frequently from some samples. It appeared that this species was located in clumps within the soil rather than fairly distributed uniformly.

Residual Root Colonization

Fusarium spp. extensively colonized residual roots within soil, particularly in areas that had not undergone pre-plant soil fumigation (table 3). On the other hand, very few roots in soil fumigated with methyl bromide/chloropicrin were colonized with *Fusarium* spp. However such roots were frequently colonized with *Trichoderma* spp. and more than 10% of the assayed root pieces were not colonized by any fungi (table 3). In general, the higher the

Fusarium colonization, the lower the *Trichoderma* colonization and vice versa.

As with the soil assays, *F. oxysporum* made up most of the *Fusarium* isolates obtained from residual roots (table 4). Only two of the morphotypes obtained from soil samples (1 and 2) were isolated from residual roots. Four other *Fusarium* species were isolated from roots as well. These were *F. equiseti, F. acuminatum* (the same species isolated from soil) and *F. sambucinum* Fuckel and *F. sporotrichioides* Sherb. (not isolated in soil assays).

Seedling Root Colonization

Roots of healthy-appearing seedlings from plots that had not been fumigated were extensively colonized by *Fusarium* spp. (table 5). In contrast, those from fumigated plots had low levels of *Fusarium*, but fairly high levels of *Trichoderma*. Also, roots of seedlings from fumigated plots had several other (unidentified) fungi colonizing their roots, but such fungi were not considered potential pathogens. *Cylindrocarpon* spp. were detected on roots of several seedlings from non-fumigated plots (table 5).

Fusarium oxysporum was the major *Fusarium* species isolated from roots of healthy seedlings (table 6). The same two morphotypes of this species (1 and 2) that were isolated from residual roots (table 5) were again isolated from healthy seedling roots. *Fusarium* species colonizing residual roots were also isolated from healthy seedling roots with two exceptions. *Fusarium solani* (Mart.) Appel & Wollenw. was isolated at very low levels from healthy seedling roots and not at all from residual roots in soil. Also, *F. sambucinum*, which was found at low levels on residual roots was not isolated from roots of healthy seedlings.

Comparisons of the Three Assay Methods

Table 7 compares the three assay methods for estimating populations of *Fusarium* and *Trichoderma* in a bare root nursery setting. If the same capital letter occurs in a particular treatment for all three assay methods, the methods were comparable in effectiveness. For example, all three methods found that methyl bromide/chloropicrin treated areas had significantly lower levels of *Fusarium* and significantly higher levels of *Trichoderma* when compared to the other three treatments. In general, comparisons within the other three treatments likewise resulted in comparable results based on the three assays. As a result, it appeared that all three assay procedures were about equally effective in determining treatment effects on *Fusarium* and *Trichoderma* populations.

DISCUSSION

Integrated pest management within forest seedling nurseries involves reducing pesticide usage to a minimum while still allowing for production of high quality seedlings (Delahaut and Koval 1994; Heinz and Parrella 1991). Reducing pesticide use is a challenge. It will require more information about populations of potential pests and how these populations may affect seedling production, including both seedling quantity and quality (Bruehl 1989). Informed decisions regarding needed pesticide applications should be based on reliable information about pest populations. Therefore, routine monitoring of these populations will be a necessary ingredient for any satisfactory integrated pest management program (Bruehl 1989; Delahaut and Koval 1994).

Fusarium spp., particularly *F. oxysporum*, is an important soilborne pest in most bareroot nurseries in western North America (Bloomberg 1976; James et al. 1991). Populations of these potential pathogens can often reach very high levels and there is often correlations between high soil *Fusarium* populations and seedling disease (James and Beall 1999, 2000; James et al. 1996). *Fusarium*-associated diseases have routinely been controlled in bare root nurseries by pre-plant soil fumigation with general biocides, particularly mixtures of methyl bromide and chloropicrin (James 1989: Marois et al. 1983; Smith and Bega 1966). However, use of methyl bromide will no longer be an option in the

near future in the United States, primarily because it contributes to deterioration of UV-protective stratospheric ozone (Shaheen 1996; World Meterological Association 1995). Other general biocide soil fumigants may replace methyl bromide, but their use may also be limited because of important environmental and worker safety concerns (Miller and Norris 1970; James 1989). Therefore, the goal of many nursery growers is to be able to produce high-quality seedlings without relying of pre-plant soil fumigation. In order to accomplish this, an integrated pest management approach will be required, which will include an increased program of monitoring for potential pathogens (Bruehl 1989), including *Fusarium* species.

Traditionally, *Fusarium* populations have been estimated from soil dilution assays (James and Beall 1999, 2000; James et al.1996). Such assays provide adequate estimates of overall populations within the soil zone where seedling growth occurs. However, these assays do not give an accurate estimate of the portion of the *Fusarium* population capable of colonizing seedling roots as well as initiating disease. To overcome this difficulty, seedling roots can be assayed for *Fusarium* colonization (James 2002a, 2002b; James and Gilligan 1988a, 1988b; James and Perez 1999). Such assays will then give a better estimate of the proportion of the soil population capable of infecting roots. Unfortunately, this still will not adequately estimate the proportion of the *Fusarium* population capable of causing disease because both pathogenic and non-pathogenic isolates are capable of colonizing root cortical cells (Bloomberg and Lock 1972; James et al. 1991). Therefore, a more reliable assay procedure for predicting disease is necessary. Such assays might include using molecular markers that are specific for pathogenic fungal strains (Appel and Gordon 1995; Gordon and Martyn 1997; Gordon and Okamoto 1992). As yet, such markers have not been developed for soil populations of *F. oxysporum*, the major pathogen, in forest nurseries.

This evaluation compared three fairly easy-to-perform assay methods for estimating *Fusarium* populations in a bare root forest nursery. In general, all three methods (soil populations, residual soil root colonization, healthy root colonization) were similar in comparing populations resulting from four different pre-plant soil treatments. They all showed that populations were greatly reduced by chemical soil fumigation, but not by either fallowing or incorporating two different *Brassica* green manure crops. Therefore, each of the three methods would probably be equally effective in estimating treatment effects on *Fusarium* populations. However, as indicated above, neither of the three would probably adequately predict disease potential.

All three methods also resulted in similar assays of *Fusarium* speciation associated with soil or root colonization. The major species, *F. oxysporum*, was found both within soil and on seedling roots at high levels. The other *Fusarium* spp. were similar in the three assays. Also, levels of *Trichoderma* that might ameliorate *Fusarium* effects (Papavizas 1985) were similar in all three assays. Therefore, the ratio of *Trichoderma* to *Fusarium* populations, as a very rough estimate of soil suppressiveness (James 2002a, 2002b) appears to be equally predictable from either soil of seedling root assays.

Past investigations (James 2002a, 2002b) have utilized seedling root colonization by *Fusarium* to compare disease control treatments, particularly when disease impacts within test plots have been low. Since the goal of most disease control treatments is to eliminate or greatly reduce *Fusarium* populations, seedling root assays seem to be a good approach to evaluate treatment efficacy. However, if current assay methods can be improved by using molecular markers for pathogenic strains, better disease prediction may be attained. Until improved methods are forthcoming, current pathogen population estimates based on either soil or root colonization by *Fusarium* spp. will be relied on.

Table 1. Soil populations of *Fusarium* and *Trichoderma* spp. as affected by pre-plant soil treatments in a ponderosa pine production field at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Treatment[1]	**Fusarium**		**Trichoderma**		T/F Ratio[4]
	Populations[2]	Percent[3]	Populations[2]	Percent[3]	
1	112a[5]	1.8	2513b	54.3	22.4
2	1286b	20.4	717a	15.5	0.5
3	3900c	61.9	775a	16.7	0.2
4	1003b	15.9	623a	13.5	0.6
Average	1575	100	1157	100	0.7

[1] Treatments: 1 = soil fumigation with methyl bromide/chloropicrin; 2 = bare fallow with periodic cultivation; 3 = winter *Brassica* (dwarf essex) green manure crop; 4 = winter *Brassica* (mustard) green manure crop.
[2] Colony-forming units/g oven-dried soil.
[3] Percent of population of the particular fungus within each treatment.
[4] Ratio of *Trichoderma* to *Fusarium* populations.
[5] Within each column, means followed by the same letter are not significantly different (P-0.05) using Tukey's HSD.

Table 2. *Fusarium* species isolated from soil within a ponderosa pine production field at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Fungus[1]	Number of Isolates Sampled	Percent Within *Fusarium* Species	Percent of All Isolates
FOXY Type 1	212	40.3	37.6
FOXY Type 2	182	34.5	32.3
FOXY Type 3	10	1.9	1.8
FOXY Type 4	123	23.3	21.8
All FOXY	527	100	93.7
FEQU	33	100	5.9
FCHL	1	100	0.1
FACU	2	100	0.3
All *Fusarium*	563	100	100

[1] FOXY = *F. oxysporum* (Types refer to morphotypes on PDA); FEQU = *F. equiseti*; FCHL = *F. chlamydosporum*; FACU = *F. acuminatum*.

Table 3. Colonization of residual roots within soil by selected fungi as affected by pre-plant soil treatments in a ponderosa pine production field at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Treatment[1]	Percent of Sampled Roots Colonized[2]				
	FOXY	All *Fusarium*	Trichoderma	Other Fungi	No Fungi
1	6.7a[3]	8.3a	65.0b	15.0a	11.7b
2	45.0b	50.8b	27.5a	23.3b	0a
3	70.8c	80.8c	18.3a	4.2a	0a
4	57.5b	62.5b	18.3a	18.3ab	0.8a
Average	45.0	50.6	32.3	15.2	3.1

[1] Treatments: 1 = soil fumigation with methyl bromide/chloropicrin; 2 = bare fallow with periodic cultivation; 3 = winter *Brassica* (dwarf essex) green manure crop; 4 = winter *Brassica* (mustard) green manure crop.
[2] Sample sizes = 120 root pieces/treatment; 480 total root pieces sampled; FOXY = *Fusarium oxysporum*; Other Fungi = unidentified fungi growing from sampled root pieces on Komada's medium; No Fungi = No fungi grew from sampled root pieces
[3] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's HSD.

Table 4. *Fusarium* species isolated from residual roots in soil within a ponderosa pine production field at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Fungus[1]	Number of Isolates Sampled	Percent Within *Fusarium* Species	Percent of All Isolates
FOXY Type 1	132	60.3	52.2
FOXY Type 2	87	39.7	34.4
All FOXY	219	100	86.6
FEQU	10	100	3.9
FACU	2	100	0.8
FSAM	5	100	2.0
FSPO	17	100	6.7
All *Fusarium*	253	100	100

[1] FOXY = *F. oxysporum* (Types refer to morphotypes on PDA); FEQU = *F. equiseti*; FACU = *F. acuminatum*; FSAM = *F. sambucinum*; FSPO = *F. sporotrichioides*.

Table 5. Colonization of roots of non-diseased 1-0 bare root ponderosa pine seedlings by selected fungi as affected by pre-plant soil treatments at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Treatment[1]	Percent of Sampled Roots Colonized[2]				
	Fusarium oxysporum	All *Fusarium*	*Trichoderma* spp.	*Cylindrocarpon* spp.	Other Fungi[3]
1	8.0a[4]	12.7a	60.7b	0a	26.7b
2	56.0b	65.3b	2.0a	40.7b	4.7a
3	92.7c	93.3c	0.7a	8.0a	0.7a
4	88.7c	94.0c	0.7a	14.0a	0a
Average	61.3	67.3	16.0	15.7	7.8

[1] Treatments: 1 = soil fumigation with methyl bromide/chloropicrin; 2 = bare fallow with periodic cultivation; 3 = winter *Brassica* (dwarf essex) green manure crop; 4 = winter *Brassica* (mustard) green manure crop.
[2] Sample sizes = 15 seedlings randomly sampled per treatment; 10 roots pieces randomly sampled per seedling; total of 600 root pieces sampled.
[3] Unidentified fungi growing from sampled root pieces on Komada's medium.
[4] Within each column, means followed by the same letter are not significantly different (P=0.05) using Tukey's HSD.

Table 6. *Fusarium* species isolated from the roots of nondiseased 1-0 ponderosa pine seedlings at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Fungus[1]	Number of Isolates Sampled	Percent Within *Fusarium* Species	Percent of All Isolates
FOXY Type 1	322	87.5	76.3
FOXY Type 2	46	12.5	10.9
All FOXY	368	100	87.2
FEQU	16	100	3.7
FACU	7	100	1.7
FSOL	2	100	0.5
FSPO	29	100	6.9
All *Fusarium*	253	100	100

[1] FOXY = *F. oxysporum* (Types refer to morphotypes on PDA); FEQU = *F. equiseti*; FACU = *F. acuminatum*; FSOL = *F. solani*; FSPO = *F. sporotrichioides*.

Table 7. Comparison of three methods of assaying for *Fusarium* and *Trichoderma* spp. at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Treatment[2]	*Fusarium* spp.			*Trichoderma* spp.		
	Soil[3]	Residual[4]	Healthy[5]	Soil[3]	Residual[4]	Healthy[5]
1	A	A	A	B	B	B
2	B	B	B	A	A	A
3	C	C	C	A	A	A
4	B	B	C	A	A	A

[1] Within each column, capital letters in table denote significant differences (P=0.05) using Tukey's HSD.
[2] Treatments: 1 = soil fumigation with methyl bromide/chloropicrin; 2 = bare fallow with periodic cultivation; 3 = winter *Brassica* (dwarf essex) green manure crop; 4 = winter *Brassica* (mustard) green manure crop.
[3] Soil population assays
[4] Assays of residual soil roots
[5] Assays of roots of healthy-appearing seedlings

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